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**Wildfire impact: natural experiment reveals differential short-term changes in soil microbial communities**

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**Highlights**

- Natural experiment compared burnt vs unburnt sites to determine wildfire impacts
- Contrasting effects in native woodland vs managed pasture soils
- Soil NH<sub>4</sub> increased post-fire in woodland soil whilst NO<sub>3</sub> increased in pasture soil
- Rapid change with greater diversity in woodland bacterial community composition

## **Abstract**

A wildfire which overran a sensor network site provided an opportunity (a natural experiment) to monitor short-term post-fire impacts (immediate and up to three months post-fire) in remnant eucalypt woodland and managed pasture plots. The magnitude of fire-induced changes in soil properties and soil microbial communities was determined by comparing (1) variation in fire-adapted eucalypt woodland vs. pasture grassland at the burnt site; (2) variation at the burnt woodland-pasture sites with variation at two unburnt woodland-pasture sites in the same locality; and (3) temporal variation pre- and post-fire. In the eucalypt woodland, soil ammonium, pH and ROC content increased post-fire, while in the pasture soil, soil nitrate increased post-fire and became the dominant soluble N pool. However, apart from distinct changes in N pools, the magnitude of change in most soil properties was small when compared to the unburnt sites. At the burnt site, bacterial and fungal community structure showed significant temporal shifts between pre- and post-fire periods which were associated with changes in soil nutrients, especially N pools. In contrast, microbial communities at the unburnt sites showed little temporal change over the same period. Bacterial community composition at the burnt site also changed dramatically post-fire in terms of abundance and diversity, with positive impacts on abundance of phyla such as Actinobacteria, Proteobacteria and Firmicutes. Large and rapid changes in soil bacterial community composition occurred in the fire-adapted woodland plot compared to the pasture soil, which may be a reflection of differences in vegetation composition and fuel loading. Given the rapid yet differential response in contrasting land uses, identification of key soil bacterial groups may be useful in assessing recovery of fire-adapted ecosystems, especially as wildfire frequency is predicted to increase with global climate change.

**Keywords:**

Environmental disturbance; bacteria; fungi; eucalypt; Australia; fire-adapted ecosystem;

## **1. Introduction**

Wildfires are notoriously unpredictable disturbances. However, fire is an important driver of ecosystem function, vegetation dynamics and nutrient cycling. The magnitude of fire impacts is determined by the interaction between the affected ecosystem, climate and the fire regime. Fire regimes are characterised by interactions between key components such as fire intensity, frequency, size, seasonality, type and severity (Flannigan et al., 2009). There has been considerable interest in understanding belowground fire impacts, especially on soil microbial communities where fire has direct and indirect impacts (Hart et al., 2005; Muñoz-Rojas et al., 2016; Neary et al., 1999). Direct effects result from heat transfer from the soil surface to lower depths, whereas indirect fire impacts are mediated by above- and below-ground interactions between plants and the soil environment. Soil heating affects soil microbial communities through cell death, causing reductions in biomass and diversity (Neary 1999; Dooley and Treseder, 2012). In contrast, greater fire-induced impacts on soil microbial communities, in terms of spatial extent and longevity, are mediated through changes to soil organic matter quality, soil moisture retention, soil pH and buffering capacity and changes in nutrient availability. Fire also impacts rhizodeposition, plant litter accumulation, and ash and charcoal content which alter nutrient cycling and soil microbial communities (Cobo-Díaz et al., 2015). The application of molecular techniques to post-fire studies is advancing our understanding of fire-induced changes on microbial communities, especially with detailed identification of the affected communities (Ferrenberg et al., 2013; Goberna et al., 2012; Mikita-Barbato et al., 2015); however, further work is required into immediate post-fire impacts (i.e. days since fire) and time to recovery.

Fire regimes in fire-adapted biomes have led to the evolution of plant fire survival traits (Bond and Keeley, 2005). These functional traits facilitate rapid (days to weeks) post-fire regeneration, and

include post-fire basal or epicormic resprouting (e.g. eucalypts) (Clarke et al., 2015; Gill, 1975); underground storage organs (e.g. acacia); and heat or smoke-stimulated flowering and seed germination (Bond and Keeley, 2005; Gill, 1975). Specific soil microbial fire adaptations have also been observed: some Australian fungi are pyrophilous and have underground storage organs which enable them to produce fruiting-bodies two days post-fire (McMullen et al., 2011). While fire-adapted systems have evolved protective mechanisms, they could still be substantially changed in the long-term, and sometimes irreparably, if predicted changes in climate and fire regime occur, i.e., increases in fire frequency combined with shorter recovery times (Flannigan et al., 2009). Furthermore, changes in management practices, such as more frequent low-intensity prescribed burning to control fuel loads, urban encroachment, and land use change place additional pressures on the ability of fire-adapted ecosystems to recover (Bardsley et al., 2015).

Because of their unpredictable nature, wildfire studies are reactive, often opportunistic, and may not have adequate control sites for comparison. The length of time since fire varies in wildfire studies, ranging from immediate and short-term (days, weeks, months) (Dannenmann et al., 2011; Ferrenberg et al., 2013; Muñoz-Rojas et al., 2016)) to longer-term (years, decades) (MacKenzie and DeLuca, 2006; Smithwick et al., 2009; Stephan et al., 2015). Investigating post-wildfire recovery and resilience also presents challenges in replication, establishing 'before-fire' baseline conditions and locating similar, but unburnt, control sites. Despite these challenges, understanding the relationships between soil properties, microbial communities and soil function at different post-fire timescales has the potential to identify early indicators of weakening ecosystem resilience and recovery in a range of land use systems.

A wildfire which overran a site that was part of a multi-year environmental monitoring study (de Menezes et al., 2015; Prendergast-Miller et al., 2015) provided an opportunity to characterise short-term changes in soil properties and soil microbial communities in managed pasture and remnant

native eucalypt woodland plots. We focused on short-term temporal variation, given the relatively rapid recovery of fire-adapted eucalypt woodland systems (Clarke et al., 2015; Gill, 1975; Shakesby et al., 2007). The objectives were to (1) monitor short-term temporal variation in soil and microbial parameters; (2) identify soil factors which related to temporal shifts in microbial communities; and (3) identify microbial groups which responded positively and negatively to fire-induced temporal change in soil properties. Finally, as it is difficult to directly ascertain the scale of fire impacts, the magnitude of fire as an environmental disturbance was determined by including a comparison of temporal (seasonal) variation at two unburnt (control) sites within the same locality as the burnt site. This study provided a rare opportunity to discuss temporal variation in the context of fire disturbance because data were also available from a sampling campaign which took place three weeks prior to the wildfire. We therefore tested the hypothesis that the temporal shift in soil properties and microbial communities would be different between burnt and unburnt sites in each land use.

## **2. Materials and Methods**

### **2.1 Study sites and sampling design**

The wildfire occurred at one of three pastoral farms (Glenrock, Bogo, Talmo) which have been previously described (de Menezes et al., 2015; Prendergast-Miller et al., 2015). A map of the study site location is provided in the Supplementary Information (Fig. S1). The naturally-occurring wildfire spread over >14000 ha of farmland which included the Glenrock farm (the burnt site). The farms at Bogo and Talmo were not affected and therefore provided unburnt pseudo-control sites for this study. The farms are within 15 km of each other and are located in the seasonally dry temperate region of New South Wales (Australia) on brown sodosols (Isbell, 2002). Glenrock is on volcanic and

sedimentary rocks of the Silurian Douro Group. Bogo and Talmo are on Mountain Creek Volcanics of the Devonian Black Range Group (Cramsie et al., 1975). As described in Prendergast-Miller et al., (2015), the main plant species in the three pasture sites was subterranean clover (*Trifolium subterraneum* L.) with some annual and perennial grasses [e.g. phalaris (*Phalaris aquatic* L.)]. The woodlands at Glenrock and Bogo consisted of remnant native woodland areas adjacent to pasture fields: the *Eucalyptus* woodland was relatively open with a native grassy understorey; the Bogo woodland had some exotic grass species. At Talmo, the pasture lay adjacent to the Burrinjuck Nature Reserve (NSW); in this remnant native woodland, *Eucalyptus* and *Acacia* tree species had a more dense cover compared to the other two woodland plots. The three study sites were located on mature (> 40 years) sheep-grazing enterprises typical of the farming landscape in rural south-eastern Australia. In this region, land clearing (by tree logging and fire) since the mid-nineteenth century, as well as soil degradation and increasing pressure on land resources has created an increasingly fragmented remnant native woodland-managed pasture landscape (Prober et al., 2002).

Monitoring sites on paired managed pasture-remnant native woodland plots were established at each farm in October 2012 (see Fig. S2). On each adjacent pasture and remnant native woodland, a plot (100 x 100 m) was gridded and 25 wireless sensor nodes were deployed (150 nodes in total). The layout of the sensor nodes was determined by spatial prediction variance (Cressie, 1993) based on the variability of soil and microbial parameters measured in de Menezes et al., (2015). The original objective of the study was to determine spatial and temporal variation in contrasting habitats using an environmental sensor network. The physical location of the nodes marked the soil sampling points to calibrate sensor- and soil-derived measurements, and the first soil samples were taken from all nodes in December 2012 (150 node samples; 25 samples per plot). The sensor nodes marked the sampling positions, and soil samples were taken within 0.5 m of the node. Due to temporal sampling, care was taken to avoid re-sampling the previous hole. Following the wildfire in January 2013, there were two sampling campaigns: (1) to collect soils at the Glenrock fire site (one

adjacent pasture-woodland plot) over a period of 4 months (up to April 2013) to determine post-fire changes; and (2) to collect soils at the three farms to determine seasonal changes in April 2013 (three adjacent pasture-woodland plots). Twenty-five soil node samples were collected from each pasture or woodland plot at each sampling time. The original sampling design provided replication at the site level for seasonal change ( $n = 3$  adjacent land uses). However, only the Glenrock site was affected by the fire and therefore, the wildfire 'treatment' was not replicated.

Soil samples were taken at all node locations within sites in December 2012 and April 2013, to allow for seasonal comparisons between burnt and unburnt sites. The wildfire burnt through the Glenrock site in early January 2013 following extreme weather conditions [air temperature 42 °C, low relative humidity and high wind speed at 80 kph; (RFS, 2013)]. Additional soil node samples were collected post-fire at Glenrock (one week, 15 January 2013; one month, 5 February 2013, which included the first post-fire rain event; and three months, 9 April 2013), to determine the impact on and dynamics of soil properties and soil microbial communities. This sampling allowed detailed temporal comparisons within the burnt site.

## 2.2 Soil sample processing

At each node for each plot, two soil cores (0-10 cm depth, 5 cm diameter) were taken and bulked. Soils were kept cool (4 °C) during transfer to the laboratory and samples were processed within 48 hr of sampling. Soil samples were broken up by hand and homogenised, and a sub-sample was flash-frozen in liquid nitrogen for molecular analyses (see below). The remaining soil sample was analysed for a range of soil properties. Soil was extracted with cold (4 °C) 0.5 M  $K_2SO_4$  (1:5 w/v ratio) (Rousk and Jones, 2010), shaken for 60 min and analysed for extractable nitrogen (N) and carbon (C) pools. Ammonium ( $NH_4^+$ -N) and nitrate ( $NO_3^-$ -N) concentrations were determined following Mulvaney (1996) and Miranda et al., (2001) respectively; free amino acid (FAA) concentrations were quantified



using the fluorimetric o-phthalaldehyde-b-mercaptoethanol (OPAME) method (Jones et al., 2002); dissolved organic C (DOC) and total dissolved N (TDN) were analysed on a total organic C (TOC) analyser (Shimadzu TOC-VCSH/CSN p TNM-1; Kyoto, Japan). Soil microbial biomass C (C<sub>mic</sub>) and N (N<sub>mic</sub>) were measured on the TOC analyser after fumigating additional soil samples with chloroform for 24 h and extracting these samples with 0.5 M K<sub>2</sub>SO<sub>4</sub> (1:5 w/v ratio) (Vance et al., 1987). Microbial biomass C and N were corrected using correction factors of 0.45 and 0.54 for C<sub>mic</sub> and N<sub>mic</sub> respectively (Brookes et al., 1985; Wu et al., 1990). Dissolved organic N (DON) was calculated as the difference between TDN and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. Available phosphorus (P) was determined by extracting soil samples with 0.5 M NaHCO<sub>3</sub> at pH 8.5 (1:100 w/v ratio) (Rayment and Lyons, 2011) and quantified using Malachite green (Irving and McLaughlin, 1990). Air-dried soil subsamples were milled and mid-infrared (MIR) spectroscopy was used to estimate soil C fractions (particulate, humic, and resistant organic C: POC, HOC, ROC respectively) using the prediction algorithms developed in Baldock et al., (2013). These prediction algorithms were developed on Australian agricultural soils (>500 samples, including those within the study region, Yass, NSW): spectra from the soils of this study fell within the calibration, and the error statistics associated with the predicted fraction were below threshold levels. Although not a direct measure of charcoal, the estimated ROC fraction is considered to be comprised of the poly-aryl C structures consistent with charred plant biomass and lignin-derived aryl C (Baldock et al., 2013). Soil pH was measured in a 1:2 soil:water suspension, and soil moisture content determined after drying at 105 °C overnight. All results are expressed on a soil dry weight basis.

## 2.3 Soil molecular analyses

### 2.3.1 Soil DNA extraction

DNA was extracted from 0.25 g of soil using the MO-BIO PowerSoil® kit following the manufacturer's protocols except that the Qiagen TissueLizer (Venlo, Netherlands) was used (full speed for 2 minutes) after the introduction of buffer C1. DNA quality and quantity was determined by Nannodrop and Quanti-iT™ Picogreen (Life Technologies™, Mulgrave, Australia).

### 2.3.2 T-RFLP processing

A T-RFLP approach was used to compare bacterial and fungal community structure before and after the fire at Glenrock (burnt site). T-RFLP analysis was also performed to compare seasonal change in bacterial community structure across the three farms studied (Glenrock, Bogo and Talmo). DNA concentration was normalised across all samples and the bacterial 16S rRNA gene and fungal ITS region were amplified using the 27f (Lane, 1991) and 519r (Lane et al., 1985) and ITS1f (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) primers respectively. The forward primers were labelled with 6-carboxyfluorescein at the 5' end. The PCR amplification products were cleaned with Agencourt® Ampure® beads (Beckman Coulter, Lane Cove, Australia) and quantified using Picogreen® dsDNA quantification kit (Life Technologies™, Mulgrave, Australia) according to the manufacturer's instructions. Twenty-five ng of PCR products were then digested with 20 A/I restriction enzyme (New England Biolabs) overnight at 37°C, followed by precipitation with 150 µl of cold 75% isopropanol (v/v) (Sigma-Aldrich, Sydney, Australia) for 30 minutes and then centrifuged at 4000 rpm for 45 minutes. PCR fragments were added to a mixture containing 9.7 µl Hi-Di™ formamide and 0.3 µl of GeneScan™ 600 LIZ size standard. The DNA was denatured at 94°C for three minutes and the fragment lengths determined by electrophoresis using an AB3031xl Genetic Analyser (Applied Biosystems, Mulgrave, Australia); the restriction fragment profiles were obtained from GENEMAPPER® (Applied Biosystems, Mulgrave, Australia). An R script was used to filter the fragment profile using the method of Abdo et al. (2006) and remove spurious baseline peaks (minimum height of 20 fluorescence units and peaks smaller than two times the standard deviation

calculated over all peaks were removed). The Interactive Binner program (Ramette, 2009) was used to bin the resulting sizing data. For bacteria, the parameters used were: minimum and maximum peak sizes of 40 and 520 bp, respectively, minimum relative fluorescence units of 0.099, window size of 2.5 bp and shift size of 0.25 bp. For fungi, peaks smaller than 40 and larger than 600 were discarded, minimum relative fluorescence units was 0.099 and a window size of 3 bp and shift size of 0.3 bp were used. Window size was selected based on inspection of the restriction fragment size profiles using the GENEMAPPER® software.

### 2.3.3 Illumina MiSeq sequencing of soil bacteria from the wildfire site

In order to determine the effect of the wildfire on soil microbial groups at the burnt site (Glenrock), we focused on bacterial community composition by sequencing the 16S rRNA amplicons. We acknowledge that fungi, protozoa and archaea will also have been affected. Eleven sample points were randomly chosen out of the 25 in each of the woodland and pasture plots at the Glenrock site (i.e. 22 samples). In total 88 DNA samples representing all sampling times (December 2012, January, February, April 2013) were sequenced using the Illumina MiSeq platform. DNA was quantified using Qubit™ (Life Technologies™, Mulgrave, Australia), and amplified using the 27f and 519r bacterial 16S rRNA primers, which were adapted to contain barcodes and the Illumina linker sequence. Equimolar amounts of DNA were added to one MiSeq flow cell. Paired-end sequencing was carried out in the Illumina MiSeq sequencer using the 500 cycle V2 kit. Paired end reads were quality checked using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), low quality regions trimmed and merged using FLASH (Magoč and Salzberg, 2011), with a 20 bp minimum overlap. Sequences < 400 bp and with homopolymers > 8 bp and ambiguities were removed in mothur (Schloss et al., 2009) resulting in a total of 11,373,687 sequences, with a mean length of 461 bp. Sequences were clustered at 97% identity threshold and chimeras removed using USEARCH/UCHIME (Edgar, 2010; Edgar et al., 2011). The resulting OTUs were classified in mothur

using the Greengenes reference files (DeSantis et al., 2006), with a confidence threshold of 60%. OTUs classified as eukaryotic, archaeal, mitochondrial or as plastid were removed as well as sequences not classified to domain level (bacteria). Rare sequences (those OTUs occurring < 100 times in the whole dataset, roughly corresponding to OTUs occurring at one sequence per sample on average) were also removed. The resulting dataset had a total of 7,737,445 sequences, 4513 OTUs, and the average, maximum and minimum numbers of sequences per sample were 87,925, 151,516 and 49,224, respectively. OTU abundance data was rarefied to 49,224 using the rarefy\_even\_depth command in the phyloseq statistical package (McMurdie and Holmes, 2013) before statistical analyses, except for DESeq2 OTU enrichment analysis for which non-rarefied data was used following the recommendations of McMurdie and Holmes (2014). Coverage of the subsampled dataset was >0.99 (Good's coverage estimator) for all samples.

### **3. Data analysis**

Our original sample design of 150 environmental sensors deployed over three paired pasture-woodland plots was set up to maximise investment and long-term spatial-temporal data capture. As the wildfire occurred after our first sample time (December 2012) we decided to continue sampling as per our original plan (25 node samples per plot) to allow comparison with pre-fire data from the same soils in one burnt and two unburnt sites. However, this inevitably meant the post-fire study at Glenrock was pseudoreplicated, which is a consequence of investigating a real-life environmental disturbance in ecology. There is a lot of debate on how to deal with pseudoreplication (e.g. Davies and Gray, 2015; Hurlbert, 1984; Millar and Anderson, 2004) which we account for below in the analyses. This was an opportunistic study, and we have used the data to describe short-term post-fire temporal changes and compare these to seasonal changes at our study sites.

### 3.1 Data pre-treatment

Glenrock wildfire data: the data comprised soil samples collected from December 2012 (pre-fire), January, February and April 2013 (post-fire) from the Glenrock woodland-pasture site which was burnt by the wildfire in January 2013. Soil properties FAA,  $\text{NO}_3^-$ , DOC and Nmic were square root and  $\text{NH}_4^+$   $\log(x+1)$  transformed to correct for skewness. For multivariate analyses, soil data were then normalised and similarity between samples calculated using Euclidean distances. Bacterial and fungal community structure (T-RFLP data) as well as bacterial community composition (genus level) data were square root transformed to reduce the contribution of dominant TRFs/OTUs and resemblance matrices created using Bray-Curtis (Clarke and Gorley, 2006).

Glenrock, Bogo and Talmo seasonal data: the data comprised soil samples collected from the three woodland-pasture sites (Glenrock, Bogo and Talmo) in December 2012 and April 2013. Soil properties FAA,  $\text{NO}_3^-$  and soil P were square root and  $\text{NH}_4^+$   $\log(x+1)$  transformed to correct for skewness. For multivariate analyses, soil property data were normalised and Euclidean distance was used for the resemblance matrix. Bacterial community structure data (T-RFLP) were square root transformed and Bray-Curtis was used for the resemblance matrix. Multivariate analyses were conducted using PERMANOVA+ software (v7; (Anderson et al., 2008)).

### 3.2 Temporal variation in soil and microbial parameters at the Glenrock wildfire site

The wildfire at the Glenrock site provided an opportunity to compare changes in the soil environment one month before and over four months post-fire. As only one site was affected, the interpretation of the analyses is limited to the affected site.

Non-linear multidimensional scaling (nMDS) plots were created to visualise the multivariate structure in soil properties, soil bacterial and fungal community structure (T-RFLP data) and soil bacterial community composition (sequence data) at Glenrock before and after the fire across both land uses (see Supplementary Info Fig. S3).

Temporal differences in soil properties, soil bacterial and fungal community structure (T-RFLP data) and soil bacterial composition (sequence data) were tested using a permutation-based multiple analysis of variance (PERMANOVA) (Table 1). PERMANOVA is a statistical technique that enables parametric modelling for factors or treatments in experimental design without implicitly assuming Euclidean distance and explicitly assuming a univariate or multivariate Gaussian distribution for the errors in the model. The use of permutations means that statistical tests can be used that do not rely on an assumed underlying distribution. The PERMANOVA tests used 9999 permutations of residuals under a reduced model, with type III partial sums of squares.

As the analysis was in response to the wildfire event at one site and not a replicated experiment, the factor 'time' was fixed (for repeated measures; (Anderson et al., 2008)), and land use was treated as a random factor to account for sampling within one site (Millar and Anderson, 2004). A two-factor crossed design was used, with time (fixed, 4 levels (Dec, Jan, Feb, April)) and land use (random, 2 levels (pasture and woodland)). PERMANOVA is sensitive to dispersions in homogeneity, therefore significant results can indicate differences due to location in multivariate space and/or dispersion (Anderson et al., 2008). Therefore, PERMDISP (a distance-to-centroid based test on multivariate dispersions) was used to test for no differences in the within-group multivariate dispersion among groups (Anderson et al., 2008), using the combined factor 'time and land use' as this interaction was significant in the PERMANOVA results (Table 1). PERMDISP is also useful to explore changes in variability, as changes in dispersion can also be used to indicate environmental stress in ecological

studies (Anderson et al., 2008). PERMDISP was performed on Euclidean (soil data) and Bray-Curtis (microbial data) resemblance matrices; the *P*-value was determined using 999 permutations.

In order to determine the magnitude of change in individual soil properties over time, pasture or woodland soil properties were tested separately by repeated measures one-way ANOVA, with sample number as subject and time as level. Where assumptions of variance were not met, the repeated measures test was performed on ranks (SigmaPlot v.13.0).

PERMANOVA indicated significant differences in microbial community structure between time and land use (Table 1), however, these patterns were masked when observed using unconstrained nMDS plots (see Supplementary Information, Fig. S3). Therefore, canonical analysis of principal coordinates (CAP) was used to quantify and visualise these differences in the Glenrock pasture and woodland soils (Anderson and Robinson, 2003; Anderson and Willis, 2003). The CAP procedure enables characterisation of sample groups, by visualising differences and assessing the distinction between groups in multivariate space (Anderson et al., 2008). Whereas nMDS is an unconstrained ordination, CAP is a constrained ordination technique which enables discrimination among groups along an axis through the multivariate data cloud (Anderson et al., 2008). In the CAP routine, we tested the *a priori* hypothesis of there being no difference in multivariate location among groups i.e. of the microbial community structure (bacterial and fungal T-RFLP) amongst the sampling time classes for each land use by constraining the ordination to those classes. The strength of the CAP result (Table S1) was determined by the trace statistic and the percentage cross-validation allocation success, as well as by obtaining a *P*-value using permutation tests (999 tests). Microbial community structure in pasture and woodland soils were then correlated to the respective soil properties using an overlay vector function (Pearson correlation, *r*). This is an exploratory tool to identify soil properties which increase or decrease with the CAP axes (Anderson et al., 2008).

Finally, land use and temporal changes in bacterial community composition (using sequenced data) at the burnt site were determined. Identification of OTU enrichment after the fire was based on the DESeq2 (Love et al., 2014) extension from the phyloseq package following the approach outlined in McMurdie and Holmes (2014). DESeq2 was run using the Wald test, with automatic filtering of low abundance OTUs, and an alpha of 0.01. Adjusted *P*-values were calculated automatically by DESeq2. The results of the DESeq2 analysis were visualised using the ggplot2 package in R (Wickham, 2009).

### 3.3 Comparison of seasonal shifts at wildfire and unburnt sites

Although the impact of the fire at Glenrock cannot be directly tested, the magnitude of temporal shifts in soil properties and bacterial community structure were compared between the three farms and discussed in the context of the fire disturbance.

Patterns in the multivariate data between site, month and land use for soil properties and soil bacterial community structure (T-RFLP data) were first explored using nMDS plots (Fig. S3). PERMANOVA was used to test for significant differences between these factors using a three-way crossed design (9999 permutations of residuals under a reduced model, with type III partial sums of squares): site (fixed, 3 levels: Glenrock, Bogo, Talmo); month (fixed, 2 levels: December 2012, April 2013); and land use (fixed, 2 levels: pasture, woodland) (Table 2). As PERMANOVA is sensitive to dispersions, the PERMDISP routine was performed using 'site-month-land use' as the group factor; the *P*-value was determined using 999 permutations (Table 2). PERMANOVA indicated significant differences in bacterial community structure between site, land use and sampling times, however, these patterns were masked when observed using unconstrained nMDS plots (see Supplementary Information, Fig. S3). Therefore, the CAP approach was used to test the hypothesis that the temporal i.e. seasonal shift (December 2012 vs. April 2013) in bacterial community structure was different between burnt (Glenrock) and unburnt (Bogo and Talmo) sites in each land use. Diagnostic results



are given in Table S1. Soil properties associated with these temporal shifts were identified using Pearson correlations. Seasonal differences to determine the magnitude of change in individual soil properties (December vs. April, i.e. the pre- and post-fire period at Glenrock) at the three farms but within the same land use were tested using a 2-way ANOVA (SigmaPlot v.13.0).

## **4. Results**

### **4.1 Changes in soil and microbial community structure at the Glenrock wildfire site**

The wildfire destroyed the monitoring site at Glenrock, and temporal samples were taken to assess the short-term variation in soil properties and microbial communities within the burnt site. Non-linear MDS plots of soil properties and bacterial and fungal community structure (T-RFLP) indicated differences in land use; in addition, temporal changes as well as an indication of increased variability were also observed in the bacterial and fungal nMDS plots (Fig. S3). PERMANOVA showed significant time x land use interactions in soil properties and microbial groups (Table 1). However, time was not significant in the soil data, and this was also inferred from the soil nMDS plot. As PERMANOVA is sensitive to dispersion, and significant effects could be due to multivariate location and/or dispersion, further tests for dispersion were conducted (PERMDISP; Table 1). For the soil data, there was no significant difference in multivariate dispersion ( $P > 0.05$ ); therefore PERMANOVA indicated a significant land use difference in soil properties which was not due to multivariate dispersion. However, differences in dispersion were evident in the bacterial ( $P = 0.02$ ) and fungal ( $P = 0.03$ ) data sets (Table 1). Further PERMDISP analyses using land use or time as factors indicated that in the microbial data sets, time did show significant dispersion (bacteria  $P < 0.001$ ; fungi  $P < 0.05$ ), but land use did not. As dispersion can be used to infer environmental stress (Anderson et al., 2008), it is

possible that the significant temporal changes in dispersion in bacterial and fungal communities were a consequence of the wildfire.

Fire-driven changes in soil properties are often associated with alterations in pH, inorganic N, labile C and charcoal (Wan et al., 2001) which are determined by fire intensity, fuel load and land use characteristics. In terms of soil properties, the temporal dynamics of the C and N pools (Fig. 1) over the pre- and post-fire period at the Glenrock fire site indicated immediate differences post-fire. One week after the fire (January 2013), pasture soil  $\text{NO}_3^-$  had increased from 8.6 to an average of 25 mg N  $\text{kg}^{-1}$ ; while pH, DOC, HOC and  $\text{N}_{\text{mic}}$  declined ( $P < 0.05$ ) in the post-fire months (Fig 1; Supplementary information Fig. S4). In the woodland soil ROC fraction increased from 7.1 mg  $\text{g}^{-1}$  before the fire to 8.4 mg  $\text{g}^{-1}$  immediately after the fire in January ( $P < 0.01$ ; Fig. 1) and remained constant thereafter. Ammonium, FAA, DON, pH, POC, DOC all increased ( $P < 0.05$ ) in the woodland soil, while nitrate which was very low pre-fire ( $\sim 1 \text{ mg N kg}^{-1}$ ) declined to negligible levels in the post-fire months (Fig. 1; Supplementary information Fig. S4). Declines were also measured in microbial biomass C and N.

Temporal differences in bacterial and fungal community structure (T-RFLP data) were visualised using the CAP approach (Fig. 2; see also CAP diagnostics Table S1). The largest shifts in microbial community structure were observed between December and January, which coincided with the immediate post-fire period, in woodland soil bacteria and for both fungi and bacteria in the pasture soil. The woodland soil fungal community showed a large shift between January and April. Close similarity in community structure was shown between December and February in pasture soil bacteria and between February and April for woodland soil bacteria. The clustering of bacterial community structure was correlated to moisture in both soil types; however, in the pasture soil, the shift in December-January was associated with pH, while in the woodland soil, this shift was associated with FAA and DOC. Temporal shifts in fungal communities were also associated with

moisture in both soil types, but the December-January shift correlated with nitrate in the pasture soil, and the January and April shift correlated with changes in DOC, FAA, Nmic and pH in the woodland soil.

## 4.2 Temporal changes in bacterial community composition at the Glenrock wildfire site

Sequencing of the bacterial 16S rRNA gene indicated that at the genus-level, bacterial community composition varied between months in both land uses ( $P < 0.05$ ; Table 1). PERMDISP analysis showed that there was no dispersion effect (Table 1). Differential abundances in bacterial community composition after the fire were identified (Fig. 3). Immediately post-fire (December vs. January), changes to bacterial composition were mostly negative. In this period, although the OTUs declined for a similar number of phyla (four and five in woodland and pasture soil respectively), the decline in OTUs in the woodland soil was greater (an 8-fold change). Throughout the monitoring period, the change in the woodland soil bacterial composition was positive and greater in magnitude, whereas in pasture soil, the change tended to be negative with a smaller magnitude and with more phyla affected. For example, April vs. December had up to a 12-fold enrichment in OTUs belonging to eight different phyla in the woodland soil (e.g. the Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Firmicutes) while the same period in the pasture soil showed a 6-fold enrichment in OTUs from four different phyla, but a 3-fold decline in OTUs from eleven phyla. Enrichment in bacterial composition seemed to occur earlier in the woodland soil (in February) compared to pasture soil where enrichment was observed in April (Fig. 3). Bacterial composition also showed contrasting patterns for the same groups: for example, immediately post-fire, the Oxalobacteriaceae (Proteobacteria) increased in the woodland soil but declined in the pasture soil. The post-fire positive change in bacterial composition in the woodland soil was mainly seen in the Firmicutes (e.g. Bacillus) and Actinobacteria. In the woodland soil, OTU enrichment rapidly increased over time, with the number of OTUs increasing from two phyla immediately post-fire to eight phyla

3-months post-fire. In the pasture soil, OTU enrichment increased from one to five phyla, but a greater number of OTUs were negatively affected.

Temporal differences in gram-negative bacteria within the order Nitrosomonadales (phylum Proteobacteria) and the gram-positive spore-forming Bacillales (phylum Firmicutes) were quantified, as they were identified from the soils studied and these orders also include N-cycling bacterial groups (Fig. 4). The Nitrosomonadales increased one week post-fire in the pasture soil; in the woodland soil, abundance was extremely low and did not change over the post-fire period. The Bacillales were more abundant in pasture soil, but showed little change over time; in contrast, in the woodland soil this group had a low abundance which increased one month post-fire after the first rain event (February 2013) but declined thereafter.

#### 4.1 Seasonal differences between burnt and unburnt sites

The analyses from the Glenrock wildfire site indicated land use as well as temporal variation in soil microbial communities and identified soil properties which correlated with changes in microbial community structure. However, the magnitude of these changes should be taken into consideration to allow an assessment of any potential 'fire' effect. Therefore, temporal differences in soil properties and bacterial community structure (T-RFLP data) were determined by comparing December 2012 and April 2013 data sets collected from Glenrock (the burnt site) and the two control unburnt sites (Bogo and Talmo).

Non-linear MDS plots indicated potential differences between site, land use and sampling time (Fig. S3). Differences between these factors were tested using PERMANOVA (Table 2). There was a significant interaction between site x land use x time for both soil properties and bacterial

community structure. PERMDISP analysis also indicated significant dispersion in both data sets. Therefore, the CAP approach was used to visualise the site and temporal differences in each land use. Seasonal differences in bacterial community structure from December 2012 to April 2013 for the three farms are shown in Fig. 5 (see Table S1 for CAP diagnostics). At the unburnt sites (Bogo and Talmo), bacterial communities showed little distinction in structure between December 2012 and April 2013 in both pasture and woodland soils. At the unburnt sites bacterial community structure was correlated with soil moisture and microbial biomass C and N contents. However, at the Glenrock burnt site bacterial community structure in both land uses between December (pre-fire) and April (three months post-fire) was more distinct in comparison to the unburnt sites, especially at Glenrock woodland. The temporal shifts in bacterial community structure at the burnt site were correlated with nitrate in the pasture soil, and with  $\text{NH}_4^+$  and DON in the woodland soil. Therefore, change in soil bacterial communities between December and April was apparently greater at the wildfire site compared to the unburnt sites and in each land use the shift was associated with different N pools.

The temporal changes in soil properties identified at the Glenrock fire site were put into context by comparing seasonal December to April differences at all three farms. Comparison of differences in individual soil properties between the three sites (Supplementary information Fig. S5) indicated significant increases between December 2012 and April 2013 at Glenrock in pasture soil  $\text{NO}_3^-$  (average April 2013 pasture soil content 23 mg N  $\text{kg}^{-1}$ ). Changes in these properties were greater than at Bogo and Talmo (average April 2013 pasture soil content 4 and 9 mg N  $\text{kg}^{-1}$  respectively). Significant increases were also observed in Glenrock woodland soil  $\text{NH}_4^+$  (average April 2013 woodland soil content 8.7 mg N  $\text{kg}^{-1}$ ) compared to Bogo and Talmo (average April 2013 woodland soil content 2.2 and 0.5 mg N  $\text{kg}^{-1}$  respectively). However, changes in other soil properties were not so dramatic when compared to the unburnt sites. For example, DON increased post-fire at Glenrock pasture and woodland: in the pasture soil, the temporal increase was greater at Bogo; in the

woodland soil, the post-fire concentration reached was similar to that measured at the Bogo unburnt site. Declines in DOC and microbial biomass N at Glenrock were also measured at the unburnt sites. The post-fire increase in Glenrock woodland soil pH (average pH 5.5 in April 2013) did not raise the pH level above that of the unburnt sites (average woodland soil pH at Bogo and Talmo 5.8 and 5.6 respectively). Soil properties such as FAA and soil C fractions (POC, ROC) showed no significant temporal change. Therefore, apart from  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , post-fire changes in most soil properties at the burnt site were similar when compared to seasonal changes in the December to April period occurring at the unburnt sites (Fig. S5).

## 5. Discussion

Post-fire wildfire studies are reactive natural experiments and may lack adequate control or unburnt sites and replication for assessing fire-induced changes. In this study, a wildfire event occurred three weeks after soil sampling at three paired native woodland-managed pasture plots, thus providing approximate pre-fire baseline conditions in soil properties and microbial community structure. As only one farm was affected (Glenrock), comparison of variation at the wildfire site with variation at two unburnt sites in the same locality provided a means to assess the magnitude of potential fire-induced changes in soil properties and microbial communities on two contrasting land uses. Importantly, shifts in bacterial community structure and changes in soil properties (especially  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) from December 2012 (pre-fire) to April 2013 (post-fire) were greater at the wildfire pasture and woodland plots compared to the unburnt plots. The shifts in bacterial community structure (T-RFLP data) at the unburnt sites were associated with soil moisture content, while both bacterial and fungal shifts (T-RFLP data) at the burnt site were associated with changes in pH and N pools i.e. higher contents of  $\text{NO}_3^-$  in pasture soil and  $\text{NH}_4^+$  in woodland soil only observed at the burnt site. Additional post-fire monitoring of the Glenrock pasture and woodland plots (one week, one

month, three months post-fire) revealed temporal shifts in bacterial and fungal community structure and bacterial community composition, as well as significant changes in soil N pools, pH, microbial biomass and ROC content which correlated with the shifts in microbial community structure. Therefore, the results suggest that the wildfire had an impact on soil properties and bacterial and fungal communities that was greater than variation driven by seasonal changes in soil moisture observed at the unburnt sites. The results also show that the magnitude of change in microbial community structure was greater than the change in soil properties. Therefore, in order to accurately capture fire-induced changes, monitoring post-fire changes belowground in fire-adapted systems should also include an assessment of impacts on soil microbial communities as soon after a fire as possible (Goberna et al., 2012; Muñoz-Rojas et al., 2016).

## 5.1 Temporal variation in bacterial and fungal community structure at the wildfire site

The impact of environmental disturbance such as fire on the survival and recolonisation of soil microbial communities is mediated through direct effects of soil heating and indirectly through fire-induced changes to pH, soil moisture retention and nutrient availability. Post-fire soil nutrients are affected by changes in SOM, litter inputs and root exudation. Soil moisture-microbial relations in post-fire soil may be affected by increased water repellency due to alterations of SOM. However, eucalypt woodland soils can be naturally water repellent, and fire can increase or decrease this phenomenon (Doerr et al., 2004; Granged et al., 2011; Shakesby et al., 2007). Therefore, post-fire microbial-plant-soil interactions are complex.

Non-spore forming fungi, protozoa and some bacteria are sensitive to soil temperatures >70 °C (Raison, 1979). Temperatures >200 °C may be required to kill some bacterial species (Neary et al., 1999). Reductions in microbial biomass C and N are typical of fire-impacted soils (Certini, 2005; D'Ascoli et al., 2005; Neary et al., 1999) and similar declines were observed at Glenrock woodland.

Microbial biomass C did not change in the pasture plot, and Docherty et al. (2012) also reported no change in microbial biomass after fire in a grassland system. D'Ascoli et al. (2005) found microbial functional diversity recovery three months after fire in a fire-adapted Mediterranean shrub land was linked to increases in autumn moisture; in drier seasons, post-fire recovery was slower. The relative similarity in pasture soil bacterial and fungal community structure after the first rain event post-fire (February 2013) to the pre-fire community structure in December 2012 also suggests that soil moisture may have been important in the recovery of microbial communities.

In general, fire has a negative impact on fungal abundance, and the magnitude of change varies with fire regime and ecosystem type (Docherty et al., 2012; Dooley and Treseder, 2012). However, fungal studies tend to focus more on forest habitats than on grassland ecotypes (Dooley and Treseder, 2012). In Australian ecosystems, determining fungal responses to fire has also focused on eucalypt habitats rather than grasslands (McMullen et al., 2011). In eucalypt woodlands, fungal responses to fire are variable and often site-specific, with fungal declines generally observed under repeated prescribed burning (Cairney and Bastias, 2007). Indeed, some Australian woodland fungi may be pyrophilous (McMullen et al., 2011), with fruit body production stimulated by fire. Consequently, we speculate that changes in fungal community structure observed in this study in both pasture and woodland soil could be related to an increase in the post-fire flush of ascomycetes, which is a typical fire response, due to post-fire spore germination, heat stimulation of spore germination, and tolerance of post-fire conditions e.g. higher pH (McMullen et al., 2011).

## 5.2 Temporal variation in bacterial community composition at the wildfire site

As well as post-fire rain events, changes in nutrient pools were associated with variation in microbial communities. Contrasting patterns in temporal N pools were observed at the Glenrock fire site: pasture soil was marked by a dramatic increase in soil  $\text{NO}_3^-$ , which became the dominant N pool;



whereas  $\text{NH}_4^+$  increased in the woodland soil. Analysis of bacterial community composition indicated very low abundance of the Nitrosomonadales at Glenrock woodland compared to pasture. This order contains bacteria associated with N cycling, especially nitrification. This community remained low in woodland soil post-fire, which suggests that this order was inherently small and was not affected by fire impacts. In contrast, the Nitrosomonadales was more abundant in the Glenrock pasture soil. Although we cannot directly attribute the abundance of the Nitrosomonadales to increased nitrification, post-fire pasture soil was also characterised by high  $\text{NO}_3^-$  content and faster nitrification rates compared to negligible rates in the woodland soil (unpublished data, Prendergast-Miller).

In the woodland soil, the greatest change in bacterial community composition was the increase in the OTUs classified to the Bacillales order from the Firmicutes phylum in the post-fire months. The Bacillales contains many spore-formers (Vos et al., 2009), which may have allowed these bacteria to recover faster. This is in agreement with previous studies that have shown both short-term (four weeks) and long-term (three years) increases in abundance of the phylum Firmicutes following fire (Cobo-Díaz et al., 2015; Ferrenberg et al., 2013).

Small post-fire declines were seen in the Bacterioidetes while Proteobacteria remained unchanged in the woodland soil. Cobo-Díaz et al. (2015) reported greater abundance of Bacterioidetes and Proteobacteria in unburned oak woodland (in the fire-adapted Mediterranean Basin) than at burnt sites. Increases in the Rhizobiaceae, Chlorobiaceae and Flavobacteriaceae were seen in the woodland soil, which could be linked to regeneration of  $\text{N}_2$ -fixing plants such as *Acacia* tree species. Post-fire increases were also observed in the Gemmatimonadetes and Actinobacteria, and Khodadad et al. (2011) showed that these bacterial groups increased in soil after six months incubation with oak and grass derived biochars (synthesised charcoal), which suggests their potential role in degradation of pyrogenic C.

### 5.3 Temporal variation in soil properties at the wildfire site

The extent of alteration in soil properties following fire disturbance is related to intrinsic site characteristics such as biogeochemistry and aboveground vegetation (land use and fuel load), which are strongly governed by season. Comparisons of the same wildfire event over different land uses are rare, however, fire intensity is known to vary with density and composition of the above-ground vegetation (i.e. fuel load) (Neary et al., 1999). The differences observed between pasture and woodland are characteristic of each land use (e.g. negligible  $\text{NO}_3^-$  in woodland soils; higher C contents in pasture soils (de Menezes et al., 2015)), and also reflect how land uses differentially respond to fire. It is likely that the fire severity varied between the two land uses studied here because of the different above ground vegetation composition and fuel load. Pasture (grass) fires tend to spread rapidly due to the homogenous vegetation, resulting in limited heat transfer to soil (Neary et al., 1999; Raison, 1979). Grass fire soil temperatures can reach 80 °C at 2.5 cm depth (Raison, 1979). Therefore, this may have moderated soil responses in the pasture compared to the woodland system. In contrast, eucalypt wildfires can expose soils to intense heat for longer periods of time as the fire moves relatively slowly through more dense and heterogeneous woodland vegetation, resulting in soil temperatures of >300 °C at 2.5 cm depth (Raison, 1979). As soil heating is an important mechanism for altering the belowground soil environment following fire activity (Neary et al., 1999), it is likely that the woodland soil was affected more than the pasture soil due to the probable greater severity of the fire that would have occurred in the woodland vegetation. The increase in woodland soil pH and ROC content and the fact that a wider variety of soil properties were associated with post-fire shifts in woodland soil microbial communities also suggest that fire severity was greater in the woodland compared to pasture soil. In comparison, the strongest shifts

(i.e. highest correlation) in pasture soil communities were associated mainly with moisture and pH changes.

At the Glenrock fire site, soil pH increased by 0.3 units in woodland soil but decreased in the pasture soil by 0.2 units. While pH changes were also observed at the unburnt sites, the only increase was at Glenrock woodland. Wildfires tend to increase soil pH, and this change is related to ash and charcoal production and their longevity in soil, which are attenuated by post-fire rain and wind (Certini, 2005; Neary et al., 1999). Soil pH is a critical soil factor as it determines the availability of plant nutrients and is a key driver of soil microbial communities, therefore, pH changes will have subsequent impacts on soil biogeochemistry. The initial increase in woodland soil pH could be related to leaching of alkaline salts from ash and charcoal (Tomkins et al., 1991) as well as organic acid denaturation (Certini, 2005). In the woodland plot, the increase in soil ROC fraction reflects the woody vegetation composition and the increase in ROC content could also have raised soil pH. It is possible that the decline in pH at Glenrock pasture was due to seasonal change rather than fire impact, as similar declines in pH were also observed at the unburnt pasture sites.

Alteration of soil N cycling is often reported following fire disturbance in a range of ecosystems (Ball et al., 2010; Dannenmann et al., 2011; DeLuca and Sala, 2006; Stephan et al., 2015), and is related to fire-induced changes in soil organic matter. Release of  $\text{NH}_4^+$  as a direct consequence of SOM combustion, and  $\text{NO}_3^-$  from subsequent SOM mineralisation, are typical post-fire responses. Contrasting patterns in temporal N pools were observed at the Glenrock site: pasture soil was marked by a dramatic increase in soil  $\text{NO}_3^-$ , which became the dominant N pool; whereas FAA and then  $\text{NH}_4^+$  increased in the woodland soil. Soil  $\text{NO}_3^-$  did not increase in the eucalypt woodland soil, although studies in other forest systems (e.g. pine, oak) often report increases in soil  $\text{NO}_3^-$  and nitrification rates following forest wildfire (Ball et al., 2010; DeLuca and Sala, 2006; Smithwick et al., 2005). The presence of charcoal may stimulate nitrification (DeLuca et al., 2006), however, there was

no change in woodland soil  $\text{NO}_3^-$  despite the increase in woodland soil ROC content. Woodland soil  $\text{NO}_3^-$  is inherently low at these sites (de Menezes et al., 2015; Prendergast-Miller et al., 2015). Low soil  $\text{NO}_3^-$  is typical of eucalypt grassy woodlands in Australia (Adams and Attiwill, 1986) but may increase with invasion of exotic annual species (Lindsay et al., 2010; Livesley et al., 2009; Prober et al., 2002). Analysis of bacterial community composition indicated very low abundance of the Nitrosomonadales at Glenrock woodland compared to pasture. However, we have no direct evidence to link abundance of this order with soil  $\text{NO}_3^-$  pools in woodland or pasture soil at Glenrock. Increases in post-fire soil nitrification rates have been linked to changes in soil conditions, such as pH, as well as changes in microbial community composition. For example, ammonia oxidiser bacteria (AOB) respond positively to post-fire nutrient dynamics (Ball et al., 2010). Although DON is the dominant N form at these sites (de Menezes et al., 2015; Prendergast-Miller et al., 2015), and organic N cycling occurs at similar rates in both land uses (Prendergast-Miller et al., 2015), it is clear that in the short-term, the post-fire pasture soil N pool was dominated by  $\text{NO}_3^-$ . In the initial weeks post-fire, pasture soil nitrifying bacteria would be able to compete for soil  $\text{NH}_4^+$  because of the absence of plant uptake, resulting in increased  $\text{NO}_3^-$  concentrations. However, the rapid increase in pasture soil  $\text{NO}_3^-$  after fire requires further investigation to confirm its biotic or abiotic origin (although grass-derived char and ash have a low N content (Raison, 1979)). Post-fire nitrification studies are largely focused on forest systems, where  $\text{NH}_4^+$  becomes the dominant inorganic N pool due to organic matter decomposition, and the release of  $\text{NO}_3^-$  is lower and tends to have an initial lag period (Prieto-Fernandez et al., 1993; Wan et al., 2001). Furthermore, differences in charcoal properties between woody and grass-based ecosystems (Krull et al., 2006) could affect grassland soil post-fire  $\text{NO}_3^-$  concentrations and nitrification rates. Excess  $\text{NO}_3^-$  would have implications for pasture vegetation regrowth, potentially favouring the return of exotic grass species (Lindsay et al., 2010; Prober et al., 2002) and affecting the balance between grass and clover ( $\text{N}_2$  fixing) species. Higher  $\text{NO}_3^-$  would also have implications for increased denitrification as well as leaching to water systems

especially in later months with the onset of winter rains (as is typical of temperate New South Wales).

#### 5.4 The magnitude of temporal change following wildfire disturbance

Immediate and short-term changes, from one week to three months post-fire, observed in this study were put into context by comparing temporal variation at Glenrock with that of the unburnt sites. At the unburnt sites, temporal shifts in bacterial community structure were different compared to the burnt site, suggesting that fire disturbance may have had an additional role in driving temporal variation at the Glenrock site. Furthermore, bacterial communities as revealed by both T-RFLP and sequencing showed immediate changes soon after the fire (relative to the pre-fire community) and soil microbial communities displayed a greater degree of change than soil properties. Temporal differences in soil properties (with the exception of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) tended to be of a similar magnitude and/or direction as the seasonal changes observed at the unburnt sites. This suggests that soil microbial indicators of post-fire recovery and resilience need to be identified in fire-adapted systems to guide assessment of monitoring schemes (Mikita-Barbato et al., 2015; Muñoz-Rojas et al., 2016). Differential abundance analysis of the soil bacterial community composition revealed differences in fire-induced change between woodland and pasture soil communities, in terms of diversity and speed of change. Bacteria in both land uses were negatively affected one week post-fire, and immediate responses, even one day post-fire, have been shown before (Goberna et al., 2012; Muñoz-Rojas et al., 2016). However, the woodland soil communities showed greater and more rapid stimulation post-fire than the pasture soil. Rapid recovery in woodland soil bacterial communities compared to pasture soil could also reflect the impact of land use change. Microbial communities in the fire-adapted native remnant woodland responded positively post-fire compared to the managed pasture site where, in broad terms, bacterial community composition tended to be

more negatively affected by the fire. The conversion of fire-adapted native woodland to managed pasture has potentially altered soil biodiversity and function, including its response to fire.

As well as short-term responses to environmental disturbance e.g. after fire events, soil microbial communities and nutrient availability also vary with diurnal and seasonal variation in moisture and temperature (Bardgett et al., 2005). Therefore, the seasonal (December to April) trends described across the three pasture-woodland sites are part of these continual temporal fluctuations and reflect plant growth dynamics and climate. At the time of this study, plant communities were transitioning from (southern hemisphere) late summer growth to autumn, a period associated with cooler temperatures, increasing moisture and slower plant growth. Therefore, it appears that the fire resulted in only a minor disturbance to seasonal patterns which are strongly controlled by temperature and moisture.

The fire-induced changes observed from this study are short-term, but post-fire ecosystem responses can have a long memory effect. In some ecosystems, the impact of fire can still be quantified several years or decades post-fire (MacKenzie and DeLuca, 2006; Smithwick et al., 2009; Stephan et al., 2015). However, an important aspect to take into account with post-fire recovery and longevity of fire impacts is the type of ecosystem involved. Australian ecosystems are fire-adapted habitats, with a range of plant and microbial mechanisms that facilitate rapid recovery (e.g. days to weeks) after wildfire events (Clarke et al., 2015; McMullen et al., 2011; Muñoz-Rojas et al., 2016), compared to the one year recovery described following a boreal forest fire (Xiang et al., 2014). Therefore, the short-term response and recovery in soil bacterial community composition within three months post-fire at the Glenrock woodland site may be due to fire adaptation mechanisms. There is a need for further research into the legacy of microbial adaptation in derived habitats such as the pasture soil (which was converted from grassy woodland) which may be negatively affected following loss of important plant traits (e.g. resprouting), resulting in the slower recovery of pasture

soil communities. Given that Australian ecosystems are fire-adapted, fire frequency will be important in determining longer-term outcomes of wildfire events, such as loss of native species, invasion of exotic species and decline in soil function (Prober et al., 2002; Tomkins et al., 1991).

## **6. Conclusion**

A natural wildfire event provided an opportunity to monitor the immediate and short-term temporal variation in soil and microbial parameters in contrasting managed and semi-natural land uses. Clear differences were observed between managed pasture and remnant native woodland plots, which could be related to fire, soil and vegetation interactions. Importantly, the magnitude of disturbance was determined by comparing post-fire variation with temporal variation at two unburnt sites that had similar vegetation, climate and soils as the burnt site. Australian native ecosystems are fire-adapted systems and plants have evolved various traits which promote rapid recovery. Soil microbial communities showed greater temporal shifts at the burnt site compared to the unburnt sites, and these shifts were related to key changes in soil N pools which were not observed at the unburnt sites. Importantly, although bacterial community composition was negatively affected in both land uses, recovery and increases in abundance and diversity were much faster in the remnant woodland soil. This suggests that fire-adapted mechanisms may have been altered following land use conversion to pasture. However, differences in fuel loading due to contrasting vegetation composition will also have played a role in determining fire impacts belowground. As the soil microbial community showed a greater magnitude of change than the measured soil properties, it is important to include detailed measures of soil microbial community structure and composition in post-fire studies.

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996 **Table and Figure captions**

997

998 **TABLES**

999 Table 1: PERMANOVA and PERMDISP results for differences between month and land use for soil  
1000 properties, bacterial and fungal community structure (T-RFLP), and bacterial community composition  
1001 (sequenced) at the Glenrock adjacent woodland-pasture plot which was destroyed by wildfire. *P*  
1002 value (*P* (perm)) is derived from 9999 permutations.

1003

1004 Table 2. PERMANOVA and PERMDISP results for differences between site, month and land use for  
1005 soil properties and bacterial community structure (T-RFLP) at three farms (Glenrock, Bogo and  
1006 Talmo) with paired adjacent woodland-pasture plots. *P* value (*P* (perm)) is derived from 9999  
1007 permutations.

1008

1009 **FIGURES**

1010

1011 Fig. 1. Temporal changes in predicted soil C fractions (A, B) and extractable soil N pools (C, D) at the  
1012 Glenrock pasture (closed symbols) and woodland (open symbols) plots. Data are means (*n* = 25) with  
1013 bars indicating  $\pm 1$  standard error. The wildfire event was in January 2013.

1014

1015 Fig. 2. Biplots showing temporal differences in community structure (T-RFLP data) for bacteria in  
1016 pasture (A) and woodland (B), and fungi in pasture (C) and woodland (D) soils at Glenrock, from  
1017 December 2012 to April 2013. The wildfire was in January 2013. All CAP axes are significant (*P* <

0.001). Soil properties correlating with the first and second CAP axes >0.3 (Pearson correlation) are shown in bold.

Fig 3. Differentially abundant OTUs after the wildfire at Glenrock pasture (A, C, E) and woodland (B, D, F) plots. The OTUs are arranged by genus on the x axis and each dot represents an OTU, colours represent phyla. Differential abundance was analysed by comparing OTU abundance in January, February and April (2013) with the pre-fire community in December 2012 using DESeq2 extension in the phyloseq package (alpha = 0.01). Comparisons in the pasture plot are January vs. December (A), February vs. December (C), April vs. December (E); in the woodland plot January vs. December (B), February vs. December (D), April vs. December (F). The y axis indicates fold change in log base 2 units. OTUs above 0 (indicated by dashed line) are considered enriched after fire, those below 0 decreased in abundance compared to December 2012. The sequence data was not rarefied as per McMurdie et al., 2014. Plots were generated using ggplot2 (Wickham et al., 2009).

Fig. 4. Boxplots representing the percentage abundance of members of the orders Nitrosomonadales (A) and Bacillales (B) in Glenrock pasture and woodland before (December 2012) and after the wildfire (January to April 2013). Upper and lower box limits represent the first and third quartiles, the upper and lower lines represent the maximum and minimum abundances and dots represent outliers. Plots were generated using ggplot2 package in R (Wickham et al., 2009).

Fig. 5. Biplots showing seasonal differences from December 2012 (summer) to April 2013 (autumn) in soil bacterial community structure (T-RFLP data) in pasture (A) and woodland (B) plots at three farms. The wildfire was at Glenrock in January 2013; Bogo and Talmo farms were unburnt. Soil

1042 properties correlating ( $r > 0.5$ ) with the first two axes are shown in bold. All CAP axes are significant

1043  $P < 0.001$ .

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1046 Table 1

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Factor	Soil properties		Bacteria		Fungi		Bacteria	
			(T-RFLP)		(T-RFLP)		(genus level)	
	Pseudo-	<i>P</i>	Pseudo-	<i>P</i>	Pseudo-	<i>P</i>	Pseudo-	<i>P</i>
	F	( <i>perm</i> )	F	( <i>perm</i> )	F	( <i>perm</i> )	F	( <i>perm</i> )
Month	1.17	0.3351	2.78	0.022	2.47	0.026	2.23	0.0381
Land use	42.87	0.0001	24.0	0.001	19.49	0.001	52.12	0.0001
Month x	6.81	0.0001	5.41	0.001	2.71	0.001	2.00	0.003
Land use								
PERMDISP								
Group	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Factor		( <i>perm</i> )		( <i>perm</i> )		( <i>perm</i> )		( <i>perm</i> )
Month x	1.1734	0.415	4.3483	0.0027	2.7648	0.0314	1.3862	0.364
land use								

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1059 Table 2

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PERMANOVA				
Factor	Soil properties		Bacterial data	
			(T-RFLP)	
	Pseudo-F	<i>P (perm)</i>	Pseudo-F	<i>P (perm)</i>
Site	19.67	0.0001	60.67	0.0001
Month	24.73	0.0001	5.14	0.0001
Land use	44.66	0.0001	14.63	0.0001
Site x month	2.83	0.0015	6.70	0.0001
Site x land use	18.83	0.0001	7.35	0.0001
Month x land use	6.85	0.0001	1.44	0.1086
Site x month x	4.16	0.0001	4.30	0.0001
land use				
PERMDISP				
Group factor	F	<i>P (perm)</i>	F	<i>P (perm)</i>
Site x month x	6.75	0.001	4.80	0.001
land use				

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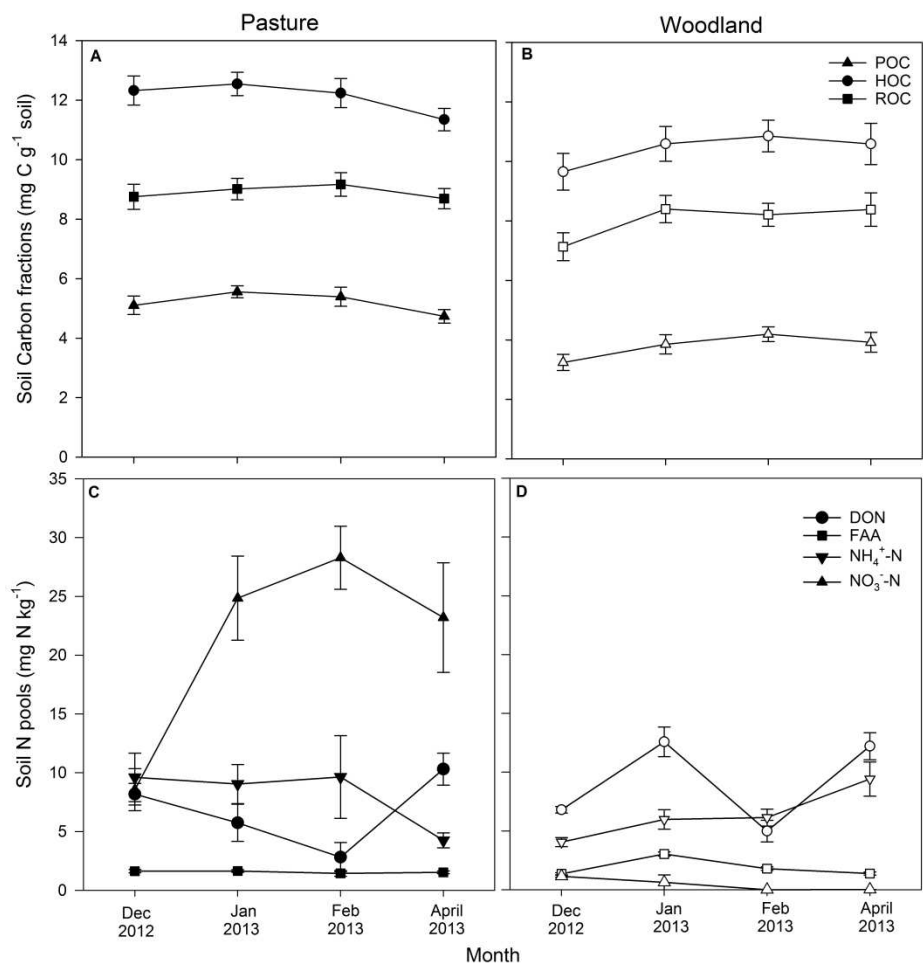
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1068 Fig 1



1083 Fig 2

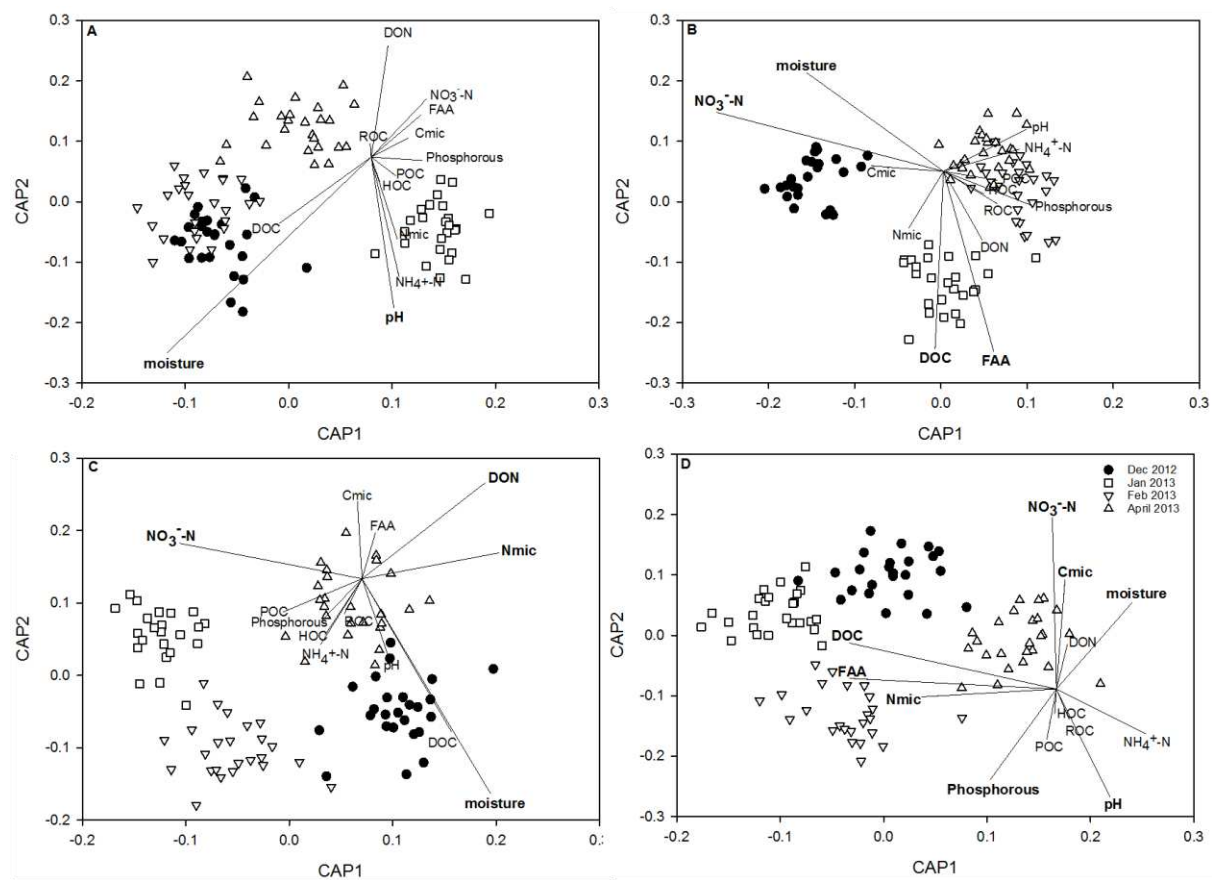




Fig 3

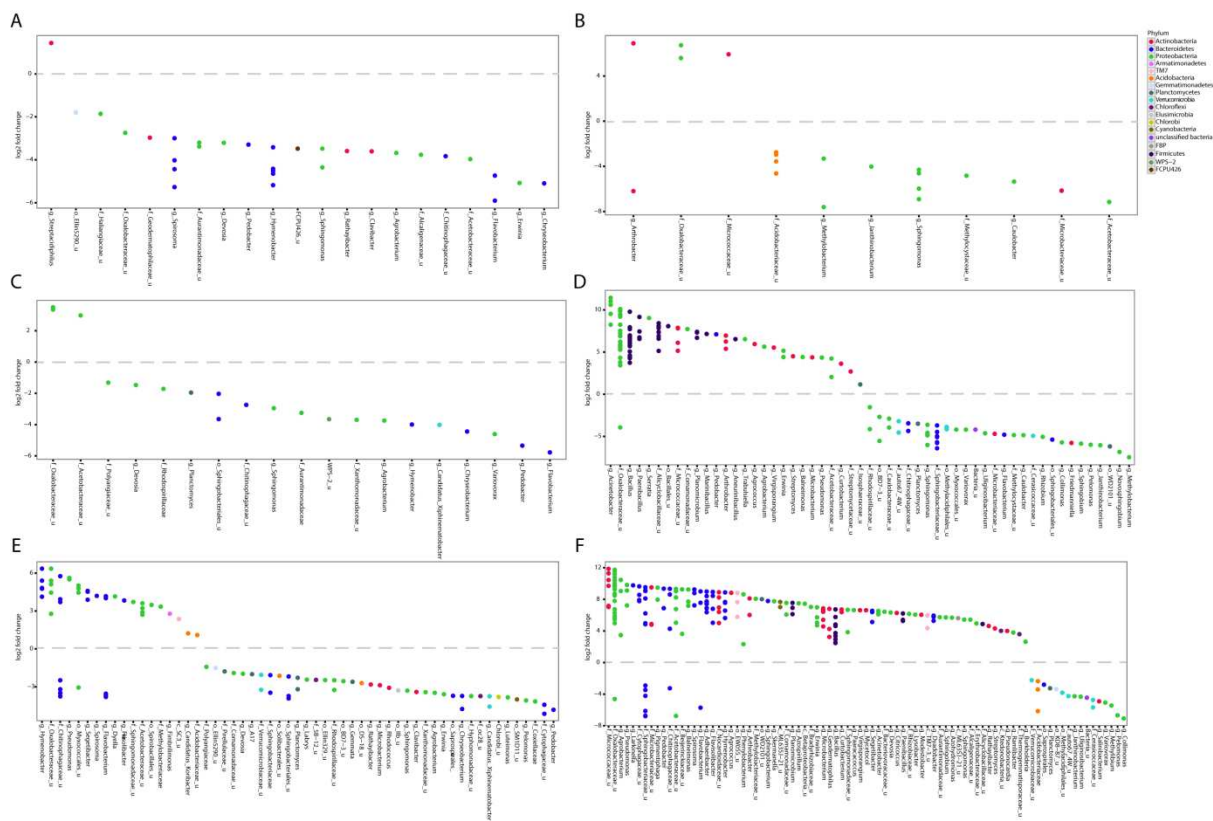


Fig 4

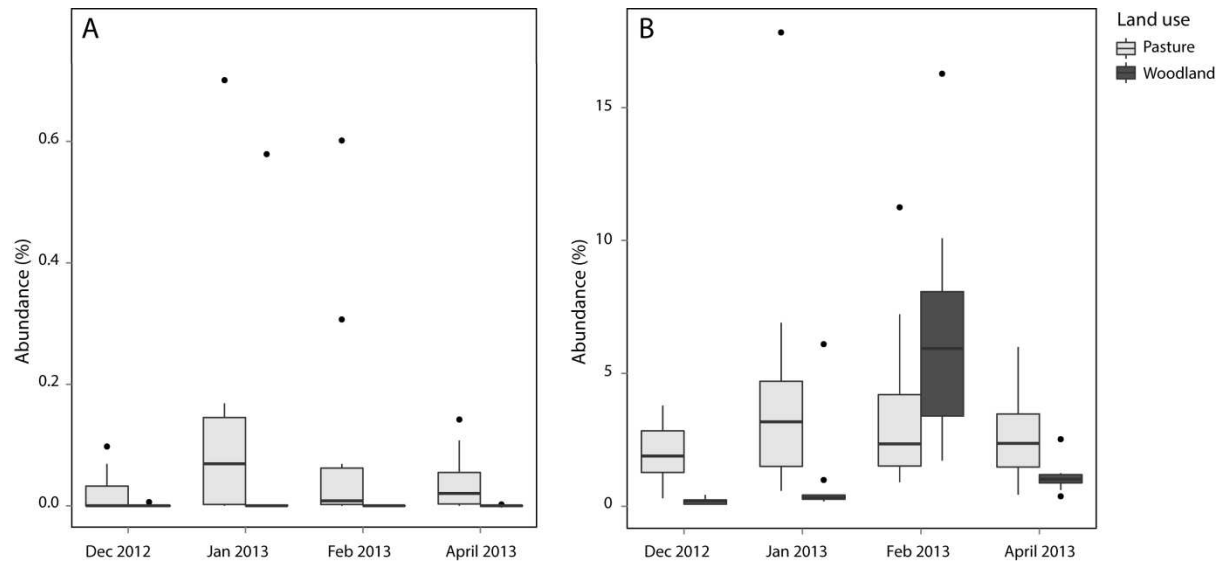
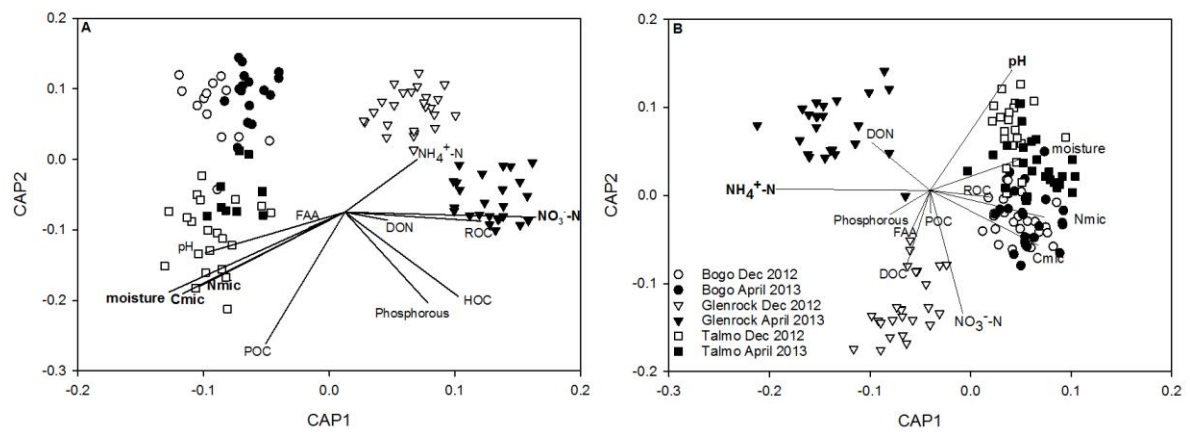


Fig 5



1134 SUPPLEMENTARY INFORMATION

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1136 **Wildfire impact: natural experiment reveals differential short-term changes in soil microbial**

1137 **communities**

1138

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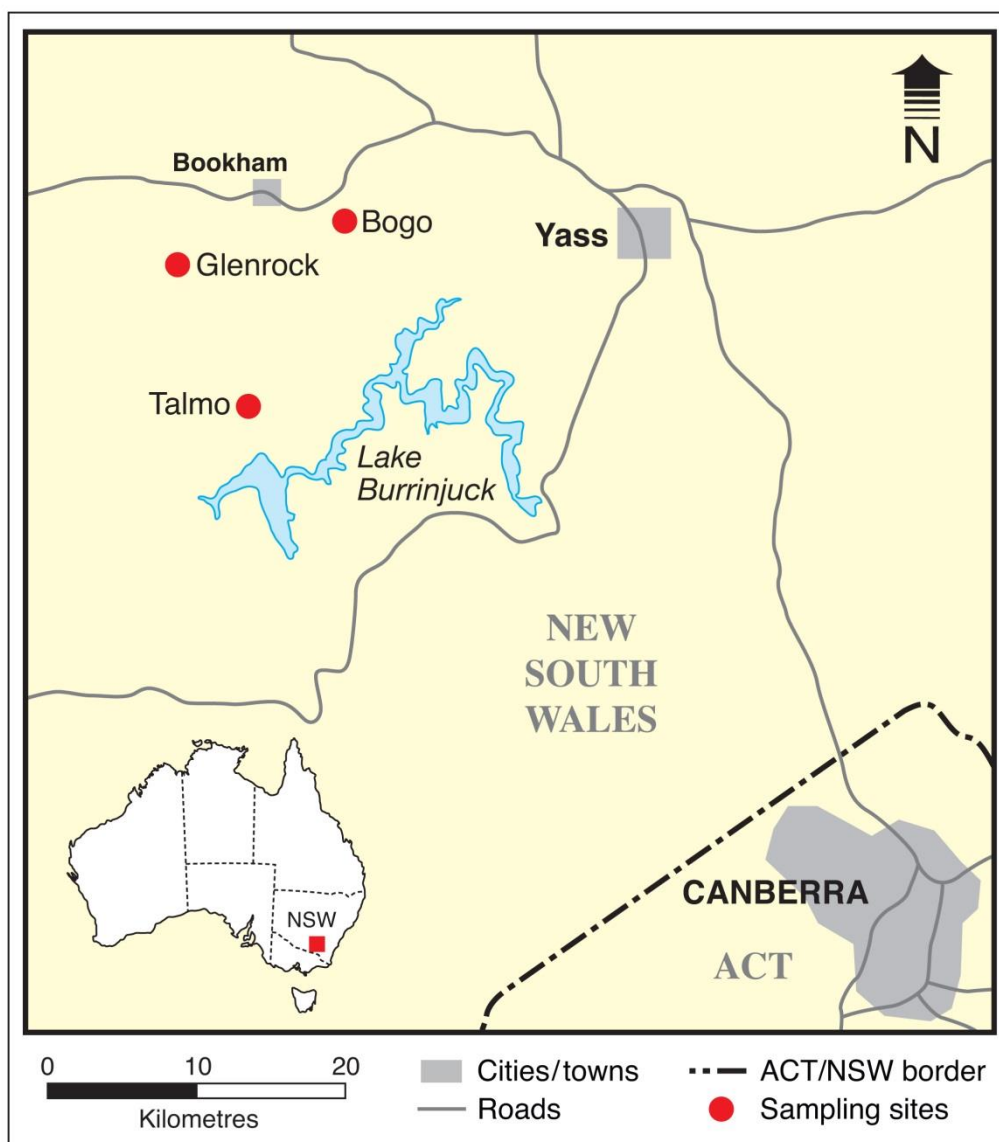


Fig. S1 Location of field sites.

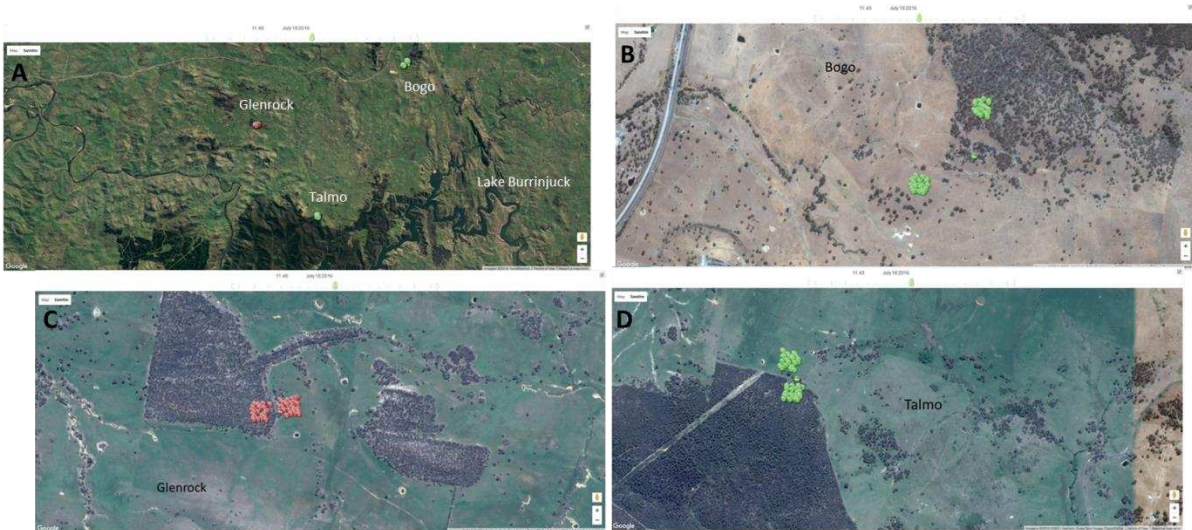
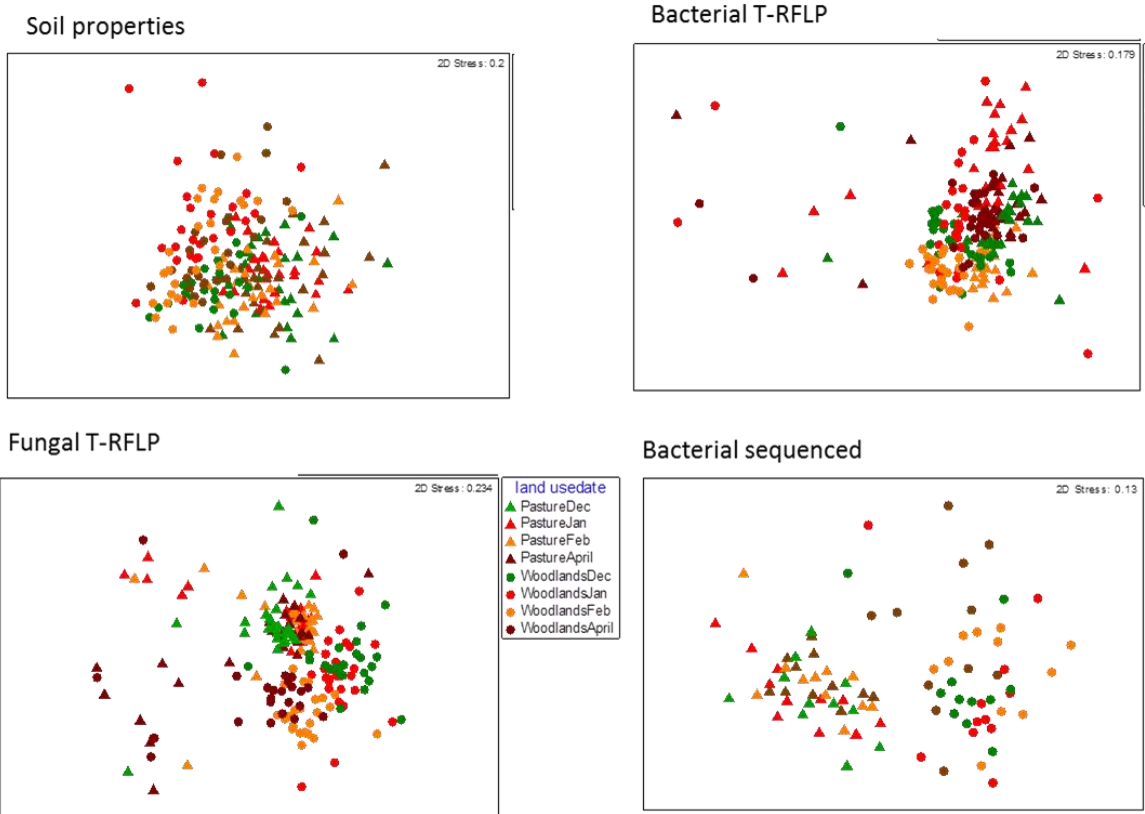


Fig. S2. Google map screen shots showing the field sites and the sensor node locations (A). Groups of 25 nodes (red and green circles) were located in 100 x 100 m plots within the remnant woodland and pasture land uses at three farms: Bogo (B), Glenrock (C) and Talmo (D). The sensor nodes were destroyed by the fire (January 2013) at the Glenrock farm (red nodes are inactive). Image taken from <http://www.sensornets.csiro.au/> (accessed July 2016).

**A: Glenrock: Temporal comparison (Dec 2012-April 2013)**



**B: Glenrock, Bogo, Talmo: Seasonal comparison (Dec 2012 & April 2013)**

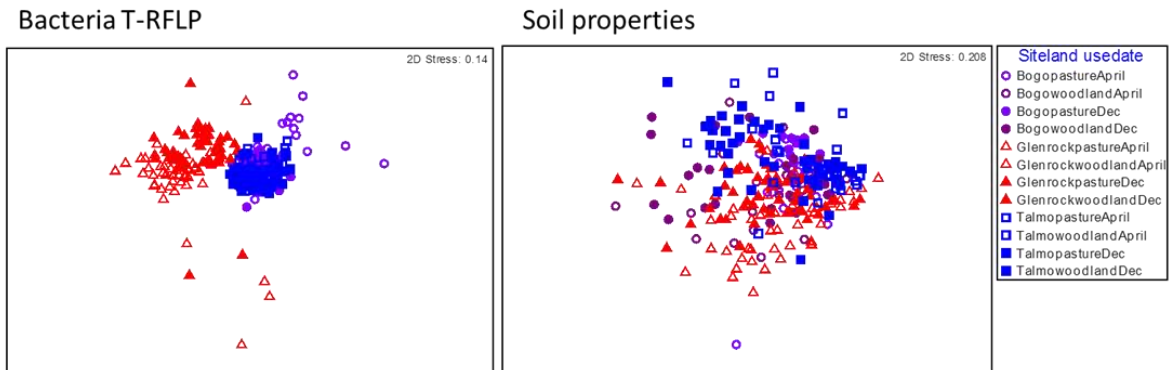
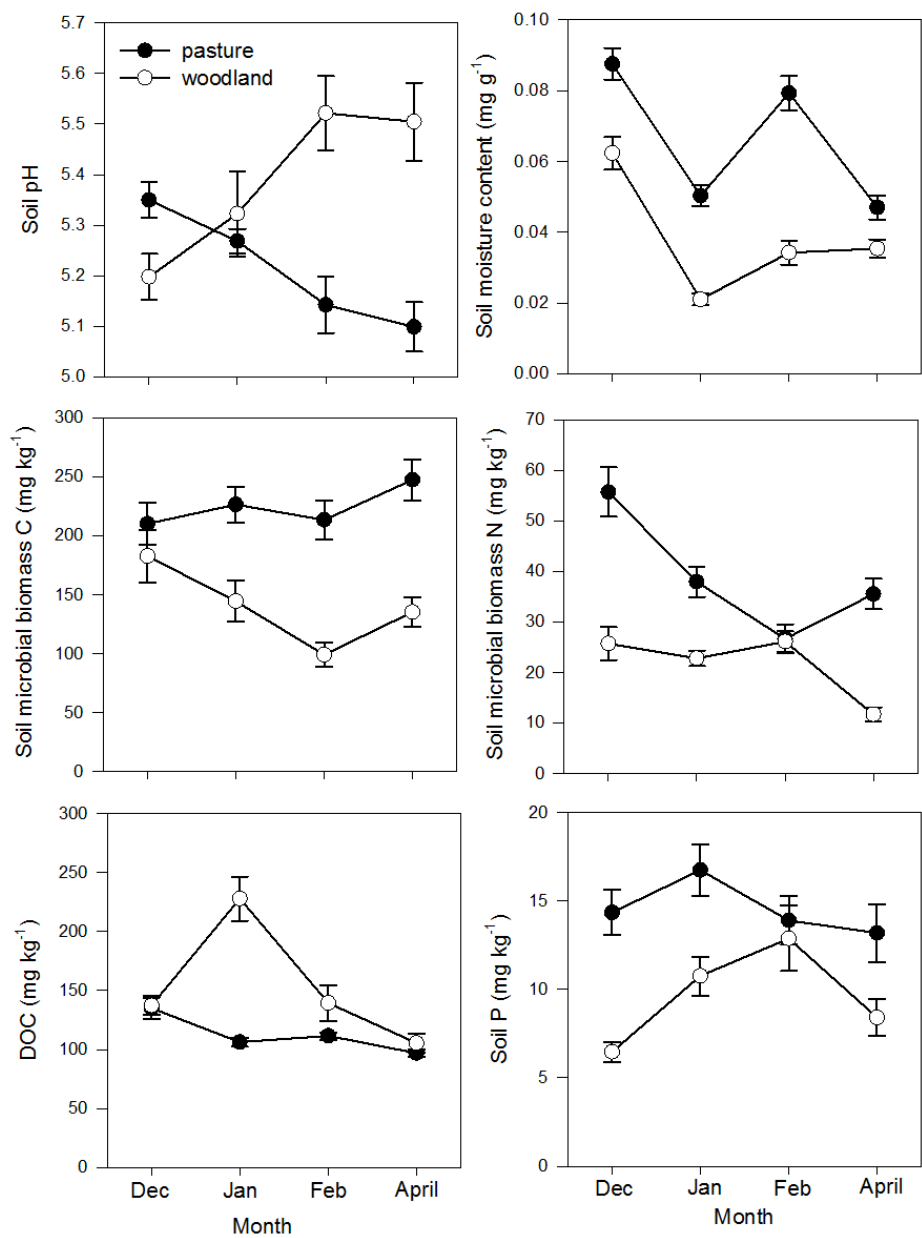


Fig. S3. nMDS plots showing unconstrained ordination of pre- and post-fire samples at (A) the Glenrock wildfire site (Dec 2012, Jan, Feb, April 2013) and (B) the seasonal comparison at Glenrock (burnt), Bogo and Talmo (unburnt sites) (Dec 2012 and April 2013).

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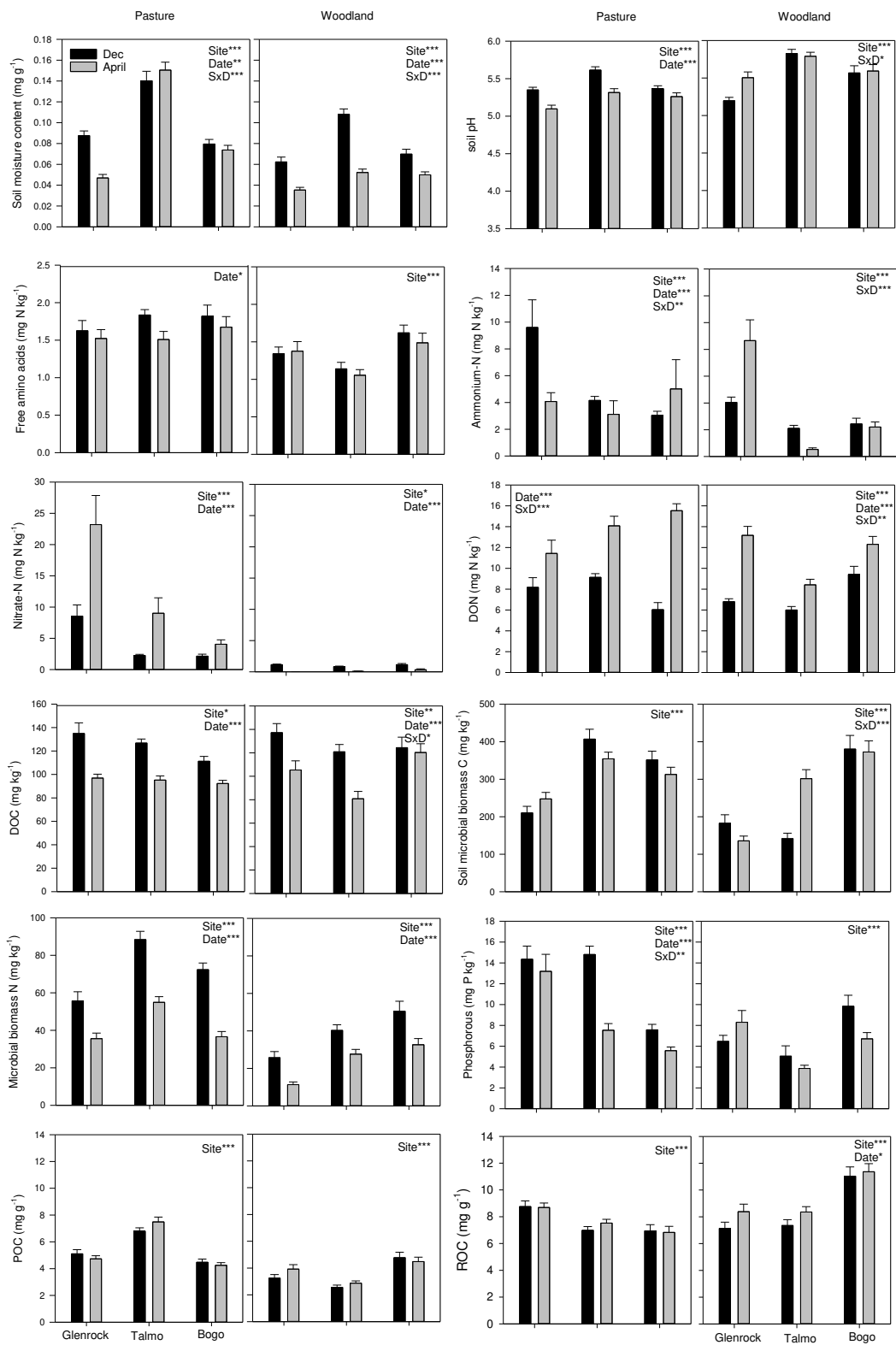
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Fig. S4. Temporal changes in soil properties at the Glenrock pasture and woodland sites, December 2012 (pre-fire) and post-fire (January, February and April 2013). Data are means ( $n = 25$ ) with bars indicating  $\pm 1$  standard error. There was a significant (at  $\alpha = 0.05$ ) effect of month on all soil properties (except for pasture soil microbial biomass C,  $P > 0.05$ ).



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Fig. S5. Comparison of soil properties from adjacent pasture and woodland plots at Glenrock (burnt site) and Talmo and Bogo (unburnt sites) in December 2012 (pre-fire) and April 2013 (three months post-fire). Data are means (n = 25) with bars indicating ± 1 standard error. Asterisks indicate significance at \*\*\* ( $P < 0.001$ ), \*\* ( $P < 0.01$ ), \* ( $P < 0.05$ ).



Table S1. CAP diagnostic statistics for analyses at the Glenrock wildfire site and for the seasonal comparison over three sites (Glenrock, Bogo and Talmo).

Site	Data set	Prop. G	Trace statistic ( <i>P</i> value)	First squared canonical correlation ( $\delta_1^2$ ) ( <i>P</i> value)	Number of PCO axes ( <i>m</i> )	Cross- validation allocation success (%)
Glenrock wildfire site  (group factor = month)	Bacteria	0.84	2.19 (0.001)	0.91 (0.001)	16	91
	T-RFLP pasture					
	Bacteria	0.78	2.52 (0.001)	0.91 (0.001)	13	98
	T-RFLP woodland					
Glenrock, Bogo and Talmo sites  (group factor = site- month)	Fungi T-RFLP pasture	0.94	2.20 (0.001)	0.88 (0.001)	34	83.5
	Fungi T-RFLP woodland	0.91	2.32 (0.001)	0.86 (0.001)	34	87.8
	Bacteria	0.99	3.17 (0.001)	0.96 (0.001)	27	81.7
	T-RFLP pasture					
	Bacteria	0.93	3.13 (0.001)	0.91 (0.001)	19	87.5
	T-RFLP woodland					

CAP analysis finds axes through multivariate data to discriminate among *a priori* groups. CAP performs a PCO analysis on the resemblance matrix and uses these to predict group membership (using discriminant analysis). In order to avoid over-parameterisation, the CAP analysis produces diagnostics to select the appropriate subset of PCO axes used in the discriminant analysis i.e. the number of axes where the probability of misclassifying a new point to the wrong group is minimised (Anderson et al 2008).

prop.G: the proportion of the variation in the data captured by the number of PCO axes selected (1.0 = 100% variation is explained)

Trace statistic: the sum of the squared canonical correlations, and the associated permutation test *P* value

$\delta_1^2$ : the size of the first squared canonical correlation and the associated permutation test *P* value

m: the number of PCO axes selected to perform the discriminant analysis

% allocation: the leave-one-out allocation success performed in the discriminant analysis

